PATENT COOPERATION TREATY

Rec'd PCT/PTO 18 APR 2005

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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File 83814
17 NOV 2004
Frank B. Dehn & Co.
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WRITTEN OPINION
(PCT Rule 66)

Date of mailing *(day/month/year)*	16.11.2004
Applicant's or agent's file reference: 27.83814	**REPLY DUE** **within 1 month(s)** from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB 03/04794	29.10.2003	29.10.2002

International Patent Classification (IPC) or both national classification and IPC
C12Q1/68

Applicant
FU, Guoliang

1. This written opinion is the **first** drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

I	☒	Basis of the opinion
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☐	Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4. For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis. For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 28.02.2005

DUE DATES NOTED
16/12/04

Name and mailing address of the international preliminary examining authority:
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I. Basis of the opinion

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed")*:

Description, Pages

1-46 as originally filed

Claims, Numbers

1-57 as originally filed

Drawings, Sheets

1-19 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   ☐ the language of publication of the international application (under Rule 48.3(b)).
   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.
   ☐ filed together with the international application in computer readable form.
   ☐ furnished subsequently to this Authority in written form.
   ☐ furnished subsequently to this Authority in computer readable form.
   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:
   ☐ the claims, Nos.:
   ☐ the drawings, sheets:

5. ☐ This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

6. Additional observations, if necessary:

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-7,22-23,35-37,39,47,50,53,54 and 57
Inventive step (IS)	Claims	1-57
Industrial applicability (IA)	Claims	-

2. Citations and explanations

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 The following **documents** are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

D1: US-A-5 744 308 (CLEUZIAT PHILIPPE ET AL) 28 April 1998 (1998-04-28)

D2: TODD ALISON V ET AL: "DzyNA-PCR: Use of DNAzymes to detect and quantify nucleic acid sequences in a real time fluorescent format" CLINICAL CHEMISTRY, AMERICAN ASSOCIATION FOR CLINICAL CHEMISTRY. WINSTON, US, vol. 46, no. 5, May 2000 (2000-05), pages 625-630, XP002245984 ISSN: 0009-9147

D3: WO 91/04340 A (CAMBRIDGE BIOTECH CORP) 4 April 1991 (1991-04-04)

2 **NOVELTY** (Art. 33(2) PCT)

2.1 D1 discloses DNA-RNA chimeric probes that are used in an amplification method. The probes comprise a single strand of a RNA polymerase promoter (template portion) , a DNA part that can hybridise to the target and leads to the RNAseH cleavage of said target (enzyme acting and target complementary portion) and a RNA part hybridising to the target that can be blocked at it 3' end (target complementary portion) (s. fig. 1, col. 8 l. 37-65, col. 11 first paragraph). The DNA-RNA chimeric probe is hybridised to the RNA target. The hybridised target is partially digested by RNAseH to allow the target molecule to be extended by DNA polymerase to complete the probe promoter. RNA polymerase uses the activated promoter to produce single stranded products (fig. 1). A similar downstream probe is used, the corresponding steps are performed and the single stranded product is again extended by the first probe to complete a cyclic reaction (col. 9).

2.2 The document D1 thus contains all the technical features of the probes claimed in independent claim 1 and dependent claims 2-7,22-23.

2.3 Furthermore document D1 discloses a method of detecting a target nucleic acid (first column). The method comprises the steps of contacting probes as disclosed in claim 1 (s. above) with a target and allowing their target complementary portion to hybridize to the target (fig. 1, col. 11), wherein the enzyme acting portion of said probe is at least partially functional (e.g. the hybridizing part that allows RNAseH activity (fig. 1, col. 11). Furthermore the method of D1 comprises the step of creating active double stranded and fully functional promoters ("enzyme acting portions", fig. 1, col. 11). The promoter activity leads to the formation of single stranded nucleic acids ("end products", fig. 1). These transcripts are again annealed to free probes and the promoter portions of said probes are rendered double stranded and fully functional (col. 13 l. 23-52). Repeating these steps (fig. 1) leads to the production of multiple copies of a single stranded nucleic acid that are detected (example 6). This detection implies an indirect detection of other reaction products.

2.4 The document D1 thus discloses all the technical features of the method claimed in independent claim 35 and dependent claims 36-37,39,47,50,53, and 54, as well as all technical features of the independent claim 57 related to a kit, since all the technical features of said kit are used in said method (s. above and col. 8-14).

2.5 Document D3 discloses primers/probes comprising one strand of a RNA polymerase promoter sequence (p. 34-35). The primers are used in a method for RNA amplification. The disclosed primer modification is suitable to act as template portion as well as as enzyme acting portions (fig. 1, 9-11). The primers/probes of D3 thus contain all technical features of the probes of claim 1.

2.6 The primers/probes of D3, that fall under the scope of claim 1 are used in a method that also involves the use of helper probes/primers (page 15, l. 15-19), the use of restriction enzymes (page 15), the use of a RNA polymerase (p. 28) implying the use of NTPs, the use of RNAseH (p. 28), the use of a DNA polymerase (p. 29) implying the use of dNTPs, buffers (p. 42) and ethidium bromide that can be regarded as detection substrate (p. 42). Consequently D3 discloses all technical features of claim 57.

2.7 In the light of D1 and D3 the subject matter of claims 1-7,22-23, 35-37,39,47,50,53,54 and 57 is not novel and does not fulfil the requirements of novelty of Article 33(2)

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3 **INVENTIVE STEP** (Art. 33(3) PCT)

3.1 The dependent claims 8-21,24-34,38,40-46,48,49,51,52,55, and 56 do not seem to contain subject matter that could lead to an inventive claim. The subject matter of said claims merely seems to represents conventional features of standard probes or detection methods that are well known to the person skilled in the art. The use of DNAzymes for the detection of amplification products is well known and e.g. disclosed in D2.

3.2 In the light of D1 the subject matter of claims 1- 57 is not inventive and does not fulfil the requirements of inventive step of Article 33(3) PCT.